changes upon reduction from Mo(VI) to Mo(IV) (George et al., 1989), and it appears that in the structure of the chicken enzyme the center is in its reduced Mo(IV) state, the protein presumably having been reduced in the strongly ionizing synchrotron beam used in data acquisition. The structure can be formulated as LMo(IV)$O(OH_2)$(S-cys), as shown in Figure 1, with the Mo=O group in the apical position of a square-pyramidal coordination geometry. It is evident from the structure of the plant enzyme that the protein is in the oxidized rather than reduced form: within the limits of the resolution of the structure, it appears to be best formulated as LMo(VI)O_2(S-cys).

Finally, a comparison of the two structures makes it possible to identify possible sites in the vertebrate protein where its heme domain might transiently dock in order to accept reducing equivalents from the molybdenum center in the course of turnover. The present work identifies a region on the surface, happily near the solvent access channel to the active site, that is conserved between all vertebrate enzymes but different from the (also conserved) sequences seen in the plant proteins. This region includes a lysine residue, but also an aspartate and glutamate, and does not seem to complement electrostatically the predominantly negatively charged surface the heme domain presents to its physiological partners. As noted by Kisker, Schwarz, and coworkers, however, it is catalytically essential that this putative docking site on the molybdenum domain not have too strong an affinity for the heme domain, as the latter must be able to swing away in order to interact with cytochrome *c* in the course of catalysis.

So we have the structure of a new molybdenum-containing enzyme, the first such from plants and the simplest yet identified from a eukaryote. The *A. thaliana* sulfite oxidase provides a unique opportunity to apply a variety of spectroscopic methods to study the molybdenum center without the complication of the strongly absorbing heme group. Its structure provides the physical context within which the electronic structure of the active site molybdenum center can be understood and correlated with reactivity. This is one of the most fundamental goals of modern bioinorganic chemistry.

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Folding Pyrin into the Family

The first structure of a pyrin domain confirms its membership in the death domain superfamily, reveals a local unfolding of the third of six helices, and suggests that the folding/unfolding transition of this helix may be an important determinant of the function and disease related dysfunction of this domain.

The death domain superfamily includes the DD (death domain), DED (death effector domain), and CARD (caspase recruiting domain) families, which mediate crucial interactions in apoptotic and developmental signaling pathways. Death domains function as protein-protein interaction motifs, serving as adaptors for bringing together components of signaling complexes. The classic example is the DD of Fas, which recruits FADD through interactions with its DD, and subsequently recruits procaspase 8 through interactions of its DED domain with that of FADD, to form the death-inducing signaling complex. Death domains only associate with their own family members, forming both homo- and heteromeric, but always homotypic interactions. Structures of two death domain heterodimers indicate that at least two different binding interfaces are possible, and models based on these structures suggest that both interfaces could be used simultaneously in a trimeric complex (Weber and Vincenz, 2001).

Pyrin domains (PYDs) were recently identified as a new family within the death domain superfamily based on a convincing combination of sequence alignment, functional association, structure prediction, and homology modeling (Bertin and DiStefano, 2000; Aravind et al., 2001; Fairbrother et al., 2001; Martinon et al., 2001; Staub et al., 2001). PYDs form homotypic protein-protein interactions and are involved in both apoptotic and inflammatory signaling pathways. Proof that PYDs are indeed members of the death domain superfamily is provided by the NMR structure, presented by Grutter, Wuthrich, and coworkers in this issue of *Structure* (Hiller et al., 2003), of the PYD of NALP1/CARD7. The PYD adopts the helical bundle Greek key motif that is characteristic of death domains. Unexpectedly, however, the region that normally corresponds to the third of the six

helices in the death domain fold is found to be unstructured. Helix 3 participates in both known death domain dimer interfaces: intimately in an Apaf-1/procaspase-9 CARD/CARD interface, and to a lesser extent in a Tube/Pelle DD/DD interface (Weber and Vincenz, 2001). An intact helix 3 is also necessary for the proper function of the Fas DD (Eberstadt et al., 1997). Therefore, the observation that helix 3 is unfolded in a wild-type pyrin domain is both surprising and significant. A further surprise is provided by a sequence alignment based on the reported PYD structure (Figure 4a of Hiller et al.), which reveals that a familial Mediterranean fever (FMF) linked point mutation, R42W, in the PYD of the protein pyrin falls within the unfolded helix 3 region.

The surprising observations reported in this paper are difficult to interpret at first glance: a disease-linked mutation is located in an unfolded region of a protein, which needs to be structured to form the protein-protein interactions required for proper function. More typically, deleterious mutations occur in structured regions and perturb well-formed structural elements that are functionally important. This indeed is the case in the lymphoproliferation mutation V238N of the Fas DD. This mutation destabilizes helix 3 in the Fas DD, which is well folded in the wild-type protein, and thereby prevents interactions with the DD of FADD (Eberstadt et al., 1997). By good fortune, this more typical case may be instructive in understanding the current results. V238 of the Fas DD forms hydrophobic interactions with residues in helices 2 and 3 of the protein. Abrogating these interactions destabilizes helix 3, which has a relatively low intrinsic helical propensity (Eberstadt et al., 1997). The hydrophobic core of the protein, however, does not involve extensive interactions with helix 3, which therefore unfolds locally, without perturbing the remainder of the protein's structure. Local unfolding of proteins upon mutation is nothing new. However, the marginal stability of helix 3 becomes more interesting with the observation that this helix is unfolded in the wild-type PYD structure. Locally unfolded regions in unmutated globular proteins are usually associated with a specific function, and often serve as conformational switches.

The possibility that helix 3 in PYDs represents a conformational switch is intriguing. Some support for such a proposition can be obtained from a comparison of the FMF linked R42W mutation in the pyrin PYD with the Fas DD. An analysis of the intrinsic helical propensity (Lacroix et al., 1998) of each helix 3 region shows that the wild-type Fas DD and the R42W PYD have a comparable intrinsic helical character, while the wild-type PYD exhibits a significant decrease in helical character. This suggests that the mutant PYD, like the wild-type DD, may possess a well-folded helix 3 stabilized by hydrophobic interactions, possibly involving W42. Helix 3 of the wild-type pyrin PYD, on the other hand, may lack sufficient helical propensity, as well as stabilizing hydrophobic interactions, and may be unfolded as observed in the mutant DD and the wild-type NALP1 PYD.

If the pyrin mutation R42W does lead to the folding of helix 3 in the PYD, how could this lead to dysfunction and disease? Protein-protein interactions that involve helix 3 of the pyrin PYD may need to be highly regulated. Local unfolding of helix 3 would effectively prevent such interactions. Under the proper conditions, for example upon formation of a complex using a different but nearby interface, helix 3 could be stabilized and could fold (as discussed by Hiller et al.), forming an intact interface and initiating the regulated interaction. The R42W mutation may drive helix 3 into the folded conformation and bypass this mechanism, leading to unregulated interactions and associated downstream events. This scenario becomes particularly attractive in light of the proposed model of trimeric death domain complexes (Weber and Vincenz, 2001). It is easy to envisage that the formation of a PYD dimer utilizing an interface with only peripheral helix 3 contacts (as in the Tube/Pelle complex) could be required for the folding of helix 3 and could be followed by interaction with a third PYD at the helix 2 and 3 interface (as in the Apaf-1 procaspase-9 complex).

Of course, many other scenarios are possible. As pointed out by Hiller et al., it remains to be determined if the absence of helix 3 is a general feature of PYDs or a specific feature of NALP1/CARD7. It is also possible that the unfolded helix 3 serves a substrate for some other protein and that the R42W mutation in pyrin exerts its effects by interfering with this interaction. These and many other possibilities, including the model above, remain to be evaluated in light of further structural and functional studies. The value of this new structure is evident in the compelling questions that it raises.

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